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Research Article

Allelopathic effect of invasive Canadian goldenrod (*Solidago canadensis* L.) on early growth of red clover (*Trifolium pratense* L.)

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Abstract

Solidago canadensis belongs to highly invasive species in Europe, it is established in ruderal, semi-natural and natural communities. To test the traits related to invasiveness, the experiment with the potential of allelopathic compounds produced by the species was conducted. The aqueous extract from the goldenrod leaves was used to examine the germination and early stages of development of *Trifolium pratense*, the species as an example of fodder plant common in meadows and pastures. Three types of aqueous extract were used: decoction, infusion, and macerate. All extracts of Canadian goldenrod had a negative influence on the germination process of red clover, however, the effect changed in time and type of extract. The most inhibiting influence was documented for the macerate type of aqueous extract. Similarly, the negative influence of macerate was the highest for the length and weight of the seedlings, the content of chlorophyll *a* and *b*, and electrolyte leakage. As the procedure of obtaining the macerate is the most like the process of natural extraction of allelopathic substances in nature, there is the practical conclusion to remove the moved biomass of the goldenrod during the restoration process of areas colonised by the species.

Keywords: allelopathy; chlorophyll; electrolyte leakage; germination; invasive species; leaves exudates

Introduction

Invasions of alien species is a worldwide serious ecological problem leading to changes in biodiversity and ecosystem functioning (Pejchar and Mooney, 2002; Pyšek and Richardson, 2010; Pyšek *et al.*, 2010). Invasions also influence a local economy (Pimentel, 2011) and they are an important task for nature conservation (Sołtys-Lelek and Barabasz-Krasny, 2010). The invasive success of non-native plants depends on their biological traits and the vulnerability of the ecosystem (Rejmánek *et al.*, 2005; Bartoszek and Stachurska-Swakoń, 2016). Natural disturbances and anthropogenic changes in environments or in management alter the possibility of invasiveness (Hobbs, 2000; Trzcińska-Tacik and Stachurska-Swakoń, 2011). Allelopathic

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compounds produced by plants are considered as an additional element facilitating competition for invasive species during settlements (Abhilasha *et al.*, 2008; Yuan *et al.*, 2013).

Solidago canadensis L. s.l. (Canadian goldenrod) is one of the successful invaders of North America origin that colonised large areas across Europe, Asia, Australia, New Zealand (Semple and Cook, 2006; Lu *et al.*, 2007; Abhilasha *et al.*, 2008; Yuan *et al.*, 2013). The species occurs in ruderal, semi-natural and natural communities, especially in moist habitats (Guzikowa and Maycock, 1986; Zając and Zając, 2015; Towpasz and Stachurska-Swakoń, 2018). It forms single-species aggregation or species-poor communities that lead to landscape homogenisation (Chen *et al.*, 2005; Yuan *et al.*, 2013), reducing diversity of native plant species (Szymura and Wolski, 2006; Szymura *et al.*, 2018) and animals, mainly insects and birds (Moroń *et al.*, 2009; Skórka *et al.*, 2010; Masło and Najberek, 2014).

Comprehension of the invasion mechanism of *S. canadensis* is an important issue in the management and recultivation of the areas colonised with the goldenrod. The species propagates vegetatively by rhizomes forming large clonal colonies and produces a huge number of small seeds easily dispersed by wind (Voser-Huber, 1983; Rosef *et al.*, 2019). The rhizomes of the species produce substances that suppress soil pathogens (Zhang *et al.*, 2009). There are few studies testing the allelopathic potential of the Canadian goldenrod (Butcko and Jensen, 2002; Sun *et al.*, 2006; Abhilasha *et al.*, 2008; Yuan *et al.*, 2013; Domaradzki *et al.*, 2017). The aim of the current experiment was to check if the allelopathic compounds from decaying biomass of goldenrod could inhibit the native species. For this experiment, meadow red clover (*Trifolium pratense* L. cv. 'Rozeta') from the Fabaceae family was selected as a fodder plant species of economic importance, due to its feed qualities. The aim of the study was to examine the effect of three types of *S. canadensis* leaf extracts in the form of decoction, infusion and macerate on seed germination and early growth of *T. pratense*. The following parameters were determined: germination indexes (1), the seedlings length (2), the fresh and dry weight and water content (3) the chlorophyll content (4) and the degree of cell membrane destabilisation by electrolyte leakage method (5).

Materials and Methods

Plant material

Trifolium pratense L. cv. 'Rozeta' (fodder, diploid cultivar, characterised by quite tall plants, prone to lying on ground) seeds were obtained from the Sadowniczy store (Poland). The *Solidago canadensis* leaves were collected in the south-eastern part of Poland - Suchoraba 49°58′37″N 20°11′49″E.

Extracts preparation

The aqueous extracts of goldenrod leave in the form of infusion (crushed dry leaves flooded with hot water), decoction (crushed dry leaves boiled in water) and macerate (crushed dry leaves flooded with water and left to stand) were prepared according to the method used by Czerwińska *et al.* (2015). The infusion was prepared from 5 g of dry *S. canadensis* leaves, which were poured over with 250 ml of boiling distilled water and left covered for 30 min. After cooling, the extract was filtered through filter papers. The decoction was prepared by weighing out 8.75 g of dry plant material, which was poured with a 1 litre of distilled water. The solution was mixed thoroughly and left for 24 h in the dark, at room temperature (20-25 °C). After one day, the aqueous extract was boiled for 15 min and filtered in the same way as the infusion. Extract in the form of a macerate was prepared by flooding 5 g of dry leaves of 100 ml cold distilled water and left in the dark, at room temperature for 24 h. After one day, the extract was filtered the same form like as infusion and decoction. The extracts were stored during the experiment at 8 °C.

Germination conditions

The Petri dishes experiment was performed as follows: clover seeds were rinsed in a 1% acetone solution, followed by distilled water and put into sterile dishes (Ø 9 cm) with three layers of filter paper (50 seeds per dish). Each Petri dish was soaked in 6 ml of the appropriate extract and watered with 3 ml of extract every other day. The control consisted of Petri dishes with seeds watered with distilled water in the same quantities as dishes with extracts. Seeds were stored in a growth chamber (Angelantoni Industrie, Italy) at 25 °C/20 °C temperature (day/night), with 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity and relative humidity RH% 60-70%, during 8 days. The experiment was carried out in 3 repetitions in 2 independent series.

Germination indexes

Germination rate (GR), germination speed (GS), allelopathic effect response index (RI) (Gao *et al.*, 2009) and seedling vigour index (SVI) (Islam *et al.*, 2009).

GR = (number of germinated seeds/ total number of seeds) × 100

GS = ((GT × D) / (GC × D)) × 100

where: GT is the number of germinated seeds daily in the treatment, GC is the number of germinated seeds daily in the control, and D is the number of corresponding days

RI = 1 – C / T (when T ≥ C)

where: C is the control germination speed and T is the treatment germination speed

SVI = (seedling length (cm) × percentage of germinated seeds) / 100.

Biometric analysis

The length of *T. pratense* (root and hypocotyl) seedlings treated the aqueous extracts from *S. canadensis* was measured using a calliper (Topex 31C615, Poland), to the nearest of 0.1 cm, after the 8 days from start of the experiment. The inhibition of percentage growth (IP), expressed as a percentage of control seedlings index, was determined according to formula used by Mominul Islam and Kato-Noguchi (2012).

Fresh and dry weight and tissue water content

Seedlings were weighted (fresh weight - FW) (Radwag WPS120, Poland) and dried (dry weight - DW) at 105 °C for 48 h in oven (Wamed SUP 100, Poland). Tissue water content (TWC) was studied according to Black and Pritchard (2002) with some modifications.

Chlorophyll content

The content of chlorophyll *a*, *b*, *a* + *b* was determined according to Barnes *et al.* (1992). The aboveground parts of the seedlings were extracted in dimethyl sulfoxide (SIGMA-Aldrich) at 65 °C for 12 hours. The absorbance of chlorophyll *a* and *b* was determined at the wavelength: λ = 665 and 648 nm, using a spectrophotometer Aquarius 9500 (Cecil Instruments, Cambridge, United Kingdom).

Electrolyte leakage

The degree of cell membranes destabilisation by the electrolyte leakage was carried out on clover seedlings according to the method described by Redmann *et al.* (1986).

Statistical analysis

The importance of variability between objects by Anova was checked. The analysis of the differences between groups was examined by Duncan test for homogeneous groups, at the level of $p \leq 0.05$; values marked with different letters (a, b, c) differ significantly. The calculations were performed using Statistica for Windows 13.1.

Results

Germination indexes

Compared to the control, in 8 days, the highest germination rate (GR) values were found in seeds grown on the *Solidago canadensis* decoction. The lowest values of germinated *Trifolium pratense* cv. Rozeta seeds was observed in Petri dishes with macerate. Germination speed (GS), expressed as a percentage of control, was significantly highest for seeds watered with decoction, and the lowest for seeds on the macerate. Allelopathic effect response index (RI) showed that decoction has a positive influence on seed germination. In turn, the infusion and macerate inhibited clover seed germination. Compared to the control, the highest inhibitory effect was observed for seeds watered with macerate. The seedling vigour index (SVI) was significantly highest for the control in relation to the extracts used. Regardless of the examined seedlings organ, the infusion and macerate resulted in a significant reduction the SVI (Figure 1, Table 1).

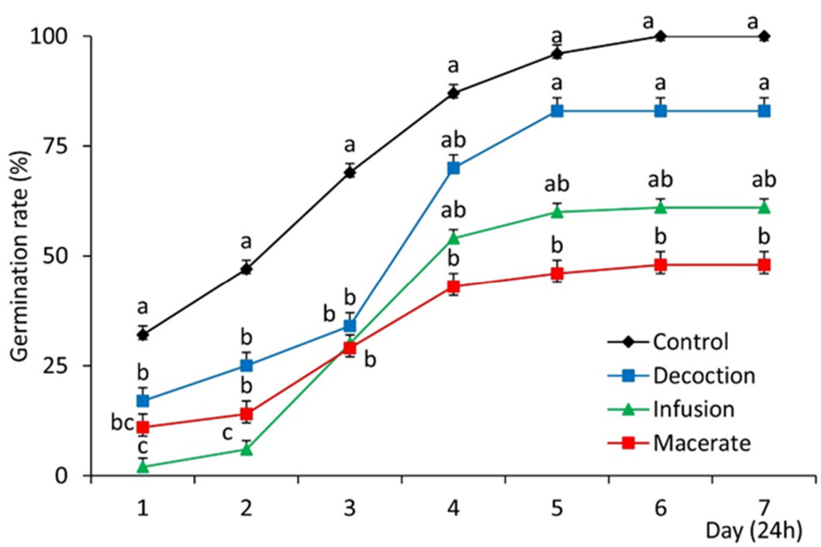

Figure 1. Germination rate (GR) of *Trifolium pratense* L. cv. 'Rozeta' seeds, treated with different types of *Solidago canadensis* L. aqueous extracts from dry leaves

Mean values (±SD) marked in different letters (a, b, c) differ significantly according to Duncan's test p ≤ 0.05

Table 1. Germination speed (GS) expressed as a percentage of control, allelopathic effect response (RI) and seedling vigour index (SVI) of *Trifolium pratense* L. cv. 'Rozeta' seeds treatment different types of *Solidago canadensis* L. aqueous extracts from dry leaves

Parameters	Control	Decoction	Infusion	Macerate
GS (%)	100	70.29 ± 0.82 a	45.06 ± 0.80 b	43.41 ± 0.84 b
RI (a.u.)	0 ± 0.00 b	0.40 ± 0.02 a	-1.08 ± 0.06 c	-1.50 ± 0.11 d
SVI whole seedling (a.u.)	2.95 ± 0.81 a	2.29 ± 0.70 a	0.28 ± 0.05 b	0.19 ± 0.07 b
SVI root (a.u.)	1.25 ± 0.33 a	1.15 ± 0.42 a	0.14 ± 0.05 b	0.08 ± 0.04 b
SVI hypocotyl (a.u.)	1.70 ± 0.54 a	1.14 ± 0.38 a	0.14 ± 0.04 b	0.11 ± 0.03 b

Note: mean values (±SD) marked in different letters (a, b, c) in row differ significantly according to Duncan's test p ≤ 0.05

Biometric analysis

Biometric analysis of *T. pratense* seedlings revealed a statistically inhibitory effect of aqueous extracts from *S. canadensis* leaves (Figure 2). On the macerate, a significant growth inhibition for the above- and underground organs of seedlings was observed. Regardless of the type of extracts, the growth of the aboveground organs was inhibited, compared to the control. Analysis of the length of whole clover seedlings showed a negative impact of each of the extracts. Compared to the control, no significant differences in length seedling growth were observed for *T. pratense* germinated on the decoction.

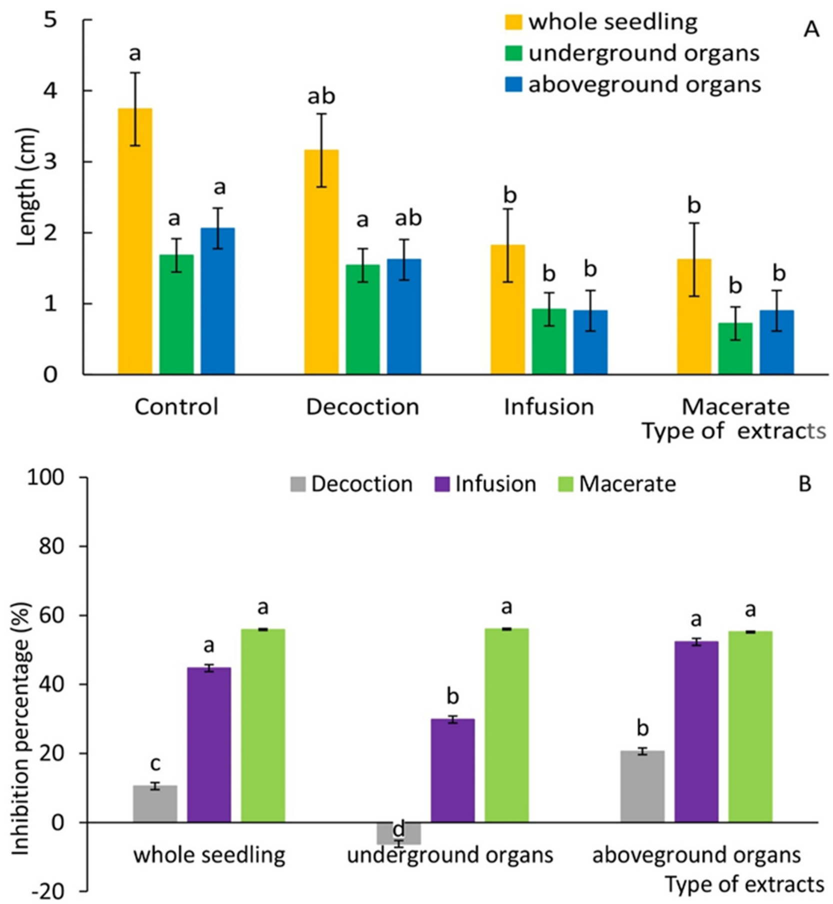

Figure 2. Length of seedlings (cm) (A) and inhibition percentage index IP (B), of *Trifolium pratense* L. cv. Rozeta seedlings, which were treated with different types of *Solidago canadensis* L. aqueous extracts from dry leaves

Mean values (±SD) marked different letters (a, b, c) differ significantly according to Duncan's test p ≤ 0.05; (B) - a negative (-) value indicates stimulation of growth, and a positive (+) value indicates growth inhibition

Fresh and dry weight and tissue water content

The fresh weight values of *T. pratense* seedlings revealed a statistically significant negative effect of extracts on biomass increase, regardless of the type of extract used. Contrary to the dry weight, for which the values increased significantly for each extract compared to the control. Water content in seedlings was similar in each treatment, compared to control (Table 2).

Chlorophyll content

In clover seedlings grown on the infusion and macerate, significant differences in the chlorophyll *a* and *b* concentration were found. In relation to the control, the smallest chlorophyll content was found in seedlings watered with macerate. Only in the case of seedlings treated with decoction no statistically significant changes in the content of the analysed pigments were observed (Figure 3).

Table 2. Fresh, dry weight and tissue water content in *Trifolium pratense* L. cv. 'Rozeta' seedlings treatment different types of *Solidago canadensis* L. aqueous extracts from dry leaves

Type of extracts	Fresh weight (g)	Dry weight (g)	Tissue water content (%)
Control	0.0156 ± 0.01 a	0.00018 ± 0.01 b	99.81 ± 0.52 a
Decoction	0.0094 ± 0.01 b	0.00045 ± 0.01 a	94.69 ± 6.14 a
Infusion	0.0097 ± 0.01 b	0.00033 ± 0.01 a	96.36 ± 3.78 a
Macerate	0.0094 ± 0.01 b	0.00032 ± 0.01 a	96.90 ± 3.35 a

Note: mean values (±SD) marked with different letters (a, b, c) in column differ significantly according to Duncan's test $p \leq 0.05$

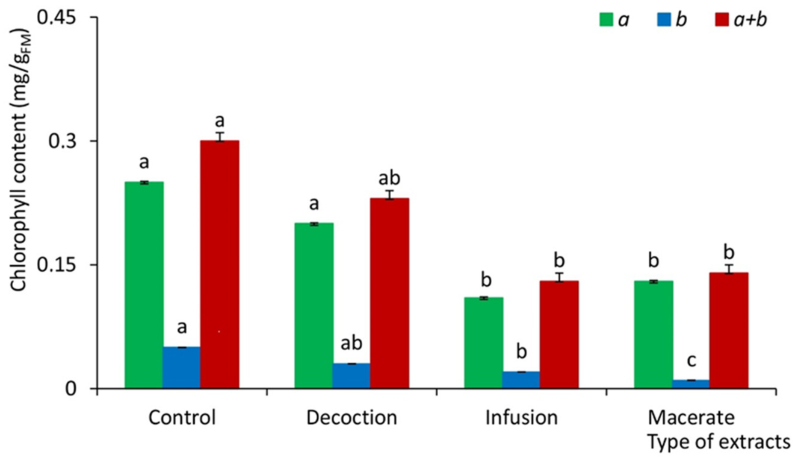

Figure 3. Content of chlorophyll *a*, *b*, *a + b* in *Trifolium pratense* L. cv. 'Rozeta' seedlings treated with different types of *Solidago canadensis* L. aqueous extracts from dry leaves

Mean values (±SD) marked with different letters (a, b, c) differ significantly according to Duncan's test $p \leq 0.05$

Electrolyte leakage

An increase of electrolyte leakage from *T. pratense* seedlings was observed in each of the *S. canadensis* extracts used. Compared to the control, the smallest changes in water and ion management were found in seedlings watered with decoction and infusion, and the largest in seedlings germinated on macerate extracts (Figure 4).

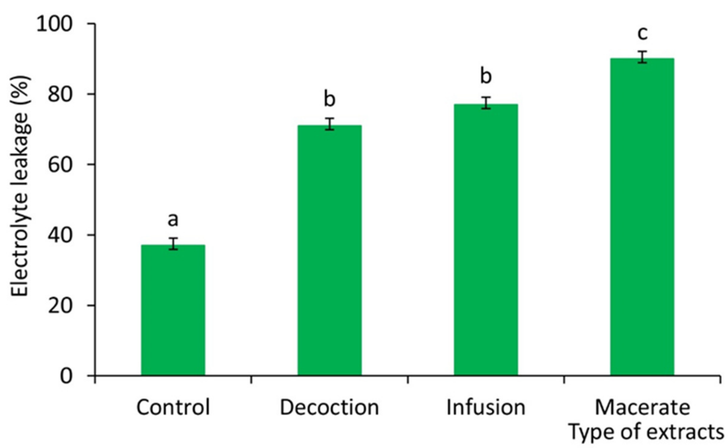

Figure 4. Electrolyte leakage in *Trifolium pratense* L. cv. Rozeta seedlings treated with different types of *Solidago canadensis* L. aqueous extracts from dry leaves

Mean values (±SD) marked with different letters (a, b, c) differ significantly according to Duncan's test $p \leq 0.05$

Discussion

Alien invasive species, similarly as local weeds, form communities with an additive system of components in which individuals compete for limited habitat resources. The effect of the competition is weakening the ability of native plants to adapt to dynamic environmental conditions (Richardson and Pyšek, 2012). This is usually manifested by inhibiting seed germination, reducing the amount of biomass produced, reducing their size, disorganising of metabolic processes in plants, etc. With a strong allelopathic interaction, even individuals die from a given type of community (Hierro *et al.*, 2003; Zandi *et al.*, 2018, 2019; Puła *et al.*, 2020).

In this experiment, aqueous extracts of *Solidago canadensis* leaves showed a pronounced inhibitory effect on the germination and growth of *Trifolium pratense* cv. 'Rozeta', in the early phase stages of development (Figure 1-2, Table 1). This confirms the known thesis that alien invasive plants release substances that cause allelopathic effects, influencing seed germination and the growth of native species (Tokarska-Guzik *et al.*, 2012; Yuan *et al.*, 2013). For example, of the 11 native species found naturally occurring in southern China, most showed sensitivity to alcoholic extracts from *S. canadensis* roots and rhizomes during germination and growth (Chen *et al.*, 2005; Yang *et al.*, 2007). However, the allelopathic effect was not observed for all species. It has been reported that *T. pratense* and *Medicago lupulina* L. react by stimulating of germination process, and during early stage of growth of red clover the stimulating of root elongation was documented. In this case, it can be concluded that root and rhizome extracts have less inhibitory properties than the various forms of leaf extracts used in the current experiment. The leaves of *S. canadensis*, as previously demonstrated, contain a wide range of compounds that may be responsible for their biological activity, among them are phenols, saponins, tannins and flavonoids (Zhao *et al.*, 2005; Zhang *et al.*, 2011). The leaves and other aboveground parts of various plants are the organs that produce the largest amount of this type of compound. The effect of allelopathic compounds on other plants may be negative in higher concentrations or positive in lower ones, as has been stated many times before (Inderjit and Duke, 2003; Bing-Yao *et al.*, 2006; Inderjit *et al.*, 2006; Sun and He, 2010; Yuan *et al.*, 2013), and the example cited above and the current experiment clearly confirm this.

Allelopathic compounds are released into the environment from plants in the form of volatile substances, by secretion from the root system into the soil, they are also washed out or released from dead parts of plants (Barabasz-Krasny *et al.*, 2017; Szafraniec *et al.*, 2019; Zandi *et al.*, 2019; Możdżeń *et al.*, 2020; Puła *et al.*, 2020). In this experiment, the negative effects of extracts varied depending on the type of extract used. Compared to the control, the highest inhibition of growth in length and weight of seedlings was found in the case of macerate, and the lowest in the presence of decoction (Figure 2, Table 2). Macerate is a kind of solution that most resembles the natural extraction of allelopathic substances from dead plant debris, occurring in nature. As the experiment showed, it contains a higher concentration of allelopathic compounds than the other extracts and significantly inhibits the development of plants. This is a very important conclusion for the management of areas colonised by Canadian goldenrod. Currently, according to recommendations, the most common way to eradicate this invasive species on large abandoned agricultural land is mowing (Kabuce and Priede, 2010). In view of the above research results, a very important here seems to be the removal of mown organic matter, which is a source of allelopathic compounds, inhibiting germination and development of native species returning to former habitats.

One of the first effects of allelopathic compounds at the cell level is membrane depolarization. It causes disorders in the transport of anions and cations, which is associated with increased permeability of these structures. Damage to membranous cell structures depends, among others on the concentration and solubility of allelopathic substances and the pH of the environment (Shann and Blum, 1987; Einhellig, 2004). Low pH values may promote the activity of allelopathic compounds released by *S. canadensis* (Wang *et al.*, 2016). In addition, the environmental stress factors increase allelochemical production and thus increase potential toxicity (An, 2005). The conducted studies showed an increase in the destabilisation of cell membranes in the presence of extracts from *S. canadensis*. Depending on the type of extract and the substances it contains, their actions resulted in a lower or higher electrolytes leakage, indicating the stress of plants treated with extracts (Figure 4). Disturbances of water-ion economy have a consequent effect on the course of other life processes of plants. For example, they play an important role in the production of chlorophyll, which is responsible for photochemical reactions. In this experiment, clover seedlings were characterised by different content of chlorophylls; the most negative effects were observed with macerate and infusion (Figure 3). Usually, when the concentration of allelopathic compounds increases in extracts, there is a significant reduction in the chlorophyll content (Puła *et al.*, 2020).

The sensitivity and reactive ability on the changes in the environment are an important mechanism of ecological invasion. An explanation in terms of physiological and ecological adaptation of alien invasive plants to the environment would certainly help to introduce effective and synthetic schemes to destruction of them. It would also facilitate forecasting potential distribution areas and estimating threats to other native flora species. To the control of invasive plants such as *S. canadensis*, attention should be paid to their scattered populations, monitored so that they are not a secondary source for seed dispersion (Hua *et al.*, 2007). It is also worth look for among native species of plants that are relatively resistant to allelopathic compounds and can pave the way for re-colonisation of areas colonised by alien invasive plants. In the light of other experiments, legumes are relatively good for this (Chen *et al.*, 2005; Yang *et al.*, 2007).

Conclusions

(1) The results presented in this study confirm allelopathic properties of various types of the aqueous extracts from *Solidago canadensis* dry leaves; chemical compounds contained in goldenrod leaves had a negative effect on *Trifolium pratense* cv. 'Rozeta' germination indexes. (2) Biometric analysis of clover seedlings revealed significant growth inhibition in the presence of infusion and macerate on *T. pratense* seedlings. (3) Each of the extracts used had a negative effect on fresh weight; the highest decrease in the value of these parameters was recorded for seedlings germinated on macerate. Dry weight was increased in all extracts, compared to the control values. (4) The aqueous extracts reduced chlorophyll content in clover seedlings; the most negative effects were caused by macerate and infusion. (5) The negative effect of aqueous extracts from *S. canadensis* leaves was confirmed by electrolyte leakage; water-ion disturbances were weaker in seedlings germinated on the decoction and infusion than in those watered with the macerate. (6) Macerate as a type of solution, is the closest to the natural extraction from dead organic debris, and its allelopathic effect is in this case the largest, so when performing treatments eliminating goldenrod, which is an important source of allelochemical compounds that inhibit germination and growth of other plants.

Authors' Contributions

Conceptualization: PZ, BBK,KM; Formal analysis: KM, BBK, PZ, AS-S; Funding acquisition BB-K; Investigation: BB-K, KM, PZ, JP; Methodology: KM, BB-K, PZ; Project administration: PZ, KM, BB-K, JP; Resources: KM, BB-K, PZ, AS-S; Software: BB-K, PZ, AS-S, JP; Visualization: PZ, KM, BB-K, AS-S; Writing - original draft: KM, BB-K, PZ, AS-S; Writing - review and editing: PZ, KM, BB-K.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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